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Transgenic Papaya: A Case for Managing Risks of *Papaya ringspot virus* in Hawaii

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Abstract
In May 1992, *Papaya ringspot virus* (PRSV) was detected in the Puna district of Hawaii Island, the main papaya growing region of the state of Hawaii. By 1994 Hawaii's papaya industry was facing devastating damage from PRSV. Efforts to develop resistant transgenic papaya were started in the mid 1980s and a resistant line was identified in 1991. Two cultivars were developed from this line and were commercialized in 1998. Rainbow, an F1 hybrid from a cross of the transgenic SunUp, and nontransgenic Kapoho are now widely planted and have helped save the papaya industry. In addition, PRSV inocula in Puna were greatly reduced as abandoned infected fields were replanted with transgenic papaya. These conditions have allowed growers to continue the production of nontransgenic Kapoho in Puna to keep the Japanese market supplied, since transgenic papaya is not yet deregulated in that country.

Introduction
Papaya (*Carica papaya*) is Hawaii's second most important fruit crop, after pineapple. Hawaii grows almost exclusively the Hawaiian Solo type and exports about 85% of its crop to the mainland USA and Japan (3). However, *Papaya ringspot virus* (PRSV), in the genus *Potyvirus*, was a potential limiting factor for growing papaya in Hawaii since the 1940s when PRSV was discovered on Oahu Island by D. D. Jensen (4), who coined the term "papaya ringspot virus." PRSV is not unique to Hawaii, and in fact is regarded as the most widespread and economically important virus that affects papaya worldwide. The severity of PRSV worldwide is due to its rapid spread by several species of aphids and to the absence of resistance in *C. papaya*. PRSV is nonpersistently transmitted by aphids, and is grouped into the PRSV-W and -P biotypes. PRSV-P infects cucurbits and papaya, whereas PRSV-W infects cucurbits but not papaya.

This article briefly summarizes the rationale, development, and commercialization of the PRSV-resistant transgenic papaya in Hawaii, and then relates the effect the transgenic papaya has had in enabling Hawaii to continue the production of nontransgenic papaya.

Papaya and PRSV in Hawaii before 1992
The Hawaiian Solo papaya was developed in Hawaii about a century ago and has been the dominant papaya for local consumption and export (3). Since the late 1950s, the dominant cultivar has been the Kapoho Solo, which typically weighs around one pound, is yellow-fleshed, sweet, and has good shipping quality. However, it is a grower-selected land race that is best adapted for the wet volcanic rocky conditions of the Puna district on Hawaii Island where Hawaii's papaya production has been focused since the mid 1960s.

Until the late 1950s and early 1960s, Hawaii's papaya production occurred mainly on the island of Oahu. However, PRSV severely affected the papaya industry in the 1950s. Subsequently, the papaya industry was relocated to Puna district on Hawaii Island in the late 1950s. Several factors caused Puna to become dominant in papaya production. These were the lack of PRSV, plenty of sunshine and high rainfall, plenty of available land that could be leased inexpensively, and the fact that papaya thrived in the volcanic lava-based soil. In fact, the papaya acreage for the state of Hawaii increased from 540 in 1957 to 2,415 in 1992, with Puna accounting for 95% of the total in 1992.

However, PRSV was a potential threat to papaya grown in Puna because it was present in the backyard plantings of households in Hilo, a city located about 19 miles away from Puna. The Hawaii Department of Agriculture (HDOA) recognized the threat and initiated surveillance teams that constantly looked for and removed PRSV in Hilo and the nearby areas. Although these practices seemed to be remarkably effective in keeping PRSV out of Puna, state agencies were nonetheless concerned about the eventual identification of PRSV in Puna. Thus, research was started in 1978 to develop control measures for PRSV in Hawaii (3).

Development and Testing of Transgenic Papaya: 1986-1998

Initial development of transgenic papaya. Efforts to develop transgenic papaya that were resistant to PRSV were started in 1986, following the breakthrough report by Beachy (7) who showed that transgenic tobacco expressing the coat protein gene of tobacco mosaic virus (TMV) were resistant or tolerant to TMV. The papaya cultivars Kapoho and Sunset were transformed with the coat protein gene of a mild nitrous acid-induced mutant of a severe PRSV strain from Hawaii (2,10). Kapoho is a yellow-flesh and was exclusively grown in Puna, whereas Sunset is red-flesh and planted in minimal acreage in Hawaii but in large quantities in Brazil. Transformation of somatic embryo cultures using a gene gun was started in 1988. Transformation and regeneration of Kapoho proved elusive, but we were able to obtain a limited number of transgenic lines of Sunset. In 1991, clones of a line (55-1) of Sunset presented resistance to greenhouse inoculations with PRSV HA (2,8). More cuttings of R0 line 55-1 were produced in tissue culture and used in a field trial that was started in April 1992 on Oahu Island.

Resistance in line 55-1 and development of transgenic cultivars. A major benefit of the 1992 R0 field trial was that it allowed us to evaluate the resistance in and the growth of the R0 plants in replicated trials and to do crosses towards developing cultivars that might be useful to the industry (5). Since Sunset and Kapoho breed true-to-type, growers normally get seeds from commercially grown fruits. As mentioned, the yellow-flesh Kapoho was the dominant cultivar, but line 55-1 was a red-flesh transgenic Sunset that was grown in small quantities in Hawaii. The transgenic Sunset, which had a single insert of the coat protein gene, was brought to homozygosity for its coat protein gene and named SunUp. However, growers in Hawaii prefer the yellow-flesh type cultivar such as Kapoho. To develop a cultivar that would be yellow flesh, virus resistant, and, hopefully, have commercially acceptable quality, an F1 hybrid of transgenic SunUp and nontransgenic Kapoho was created (6). This F1 hybrid was named Rainbow.

PRSV Identified in Puna Causing Crisis in the Papaya Industry: 1992-1998

Rapid spread of PRSV in Puna results in severe losses in papaya industry. Coincidentally, PRSV was discovered in Puna on Hawaii Island in May 1992. The long anticipated invasion of PRSV into Puna had occurred, which made our decision to set up a field trial even more critical since we had developed a possible solution to the problem. With the Puna area growing 95% of Hawaii's papaya, the devastation that PRSV could do to the industry was obvious. Immediate and large-scale actions were carried out to suppress the spread of PRSV. Initial massive cutting of trees and cooperative programs of tagging infected trees by HDOA officials followed by cutting of the trees by growers only slowed the inevitable spread. By October 1994, the virus was widespread and efforts to contain the virus were abandoned, thus causing an even faster spread of the virus. The Kapoho area, which accounted for one third of the production area in Puna, was completely infested making it impossible to economically raise papaya.

Field trial in Puna and commercialization of transgenic papaya. In 1995, a large field trial led by Steve Ferreira of the University of Hawaii was set up in a farm in Puna where PRSV had resulted in the farmer to abandon growing papaya on the farm. The trial consisted of replicated blocks and a large block of Rainbow papaya; this would serve to simulate commercial production and allow researchers, farmers, and packers to assess the quality, productivity, and acceptability of the fruit. The trial also helped obtain data that were requested by regulators, such as the spread of the transgene to border rows of the field trial and to papaya in abandoned fields that were far removed from the test site. The field trial demonstrated beyond a doubt, that Rainbow and SunUp were resistant to PRSV under intense virus pressure. Data on the field trial were taken for two and one half years during which time no breakdown in resistance of the test transgenic trees was observed. The yield and quality of Rainbow was exceptional, amounting annually to 125,000 pounds of papaya per acre as compared to the infected nontransgenic controls, which produced 5,000 pounds per acre per year (1).

Efforts to deregulate the papaya were started in late 1995. APHIS deregulated the papaya in November 1996, EPA in August 1997, and FDA completed its consultation in September 1997. Our efforts in developing the transgenic papaya, testing them in the field, and deregulating the papaya were transparent and for the sole purpose of moving as prudently as possible towards evaluating and eventually releasing a product to help save the papaya industry, and these efforts stimulated no public out-cry.

The task of obtaining the licenses for the components of the papaya that were covered by patents were turned over to the PAC (Papaya Administrative Committee), which is composed of papaya growers who have organized themselves under a USDA marketing order and who pay an assessment fee for each pound of papaya they sell. Fortunately, these efforts went well and the necessary licenses were obtained by April 1998.

Impact of Transgenic Papaya on Control of PRSV and on Growing Nontransgenic Kapoho in Puna

Reclamation of papaya-growing regions in Puna. Seeds from transgenic papaya were distributed free to growers starting May 1, 1998. The variety Rainbow comprised the overwhelming amount of the distributed seeds. By late 1998, many previously abandoned fields were reclaimed and new sites were planted; the transgenic papaya had halted the decline of the papaya industry. The transgenic papaya showed excellent resistance to PRSV, even when planted next to heavily infested fields. Harvesting of Rainbow started in 1999, and grower, packer, and consumer acceptance was widespread. The following statistics bear-out the impact of PRSV on reducing papaya production and, conversely, the impact that the transgenic papaya had on increasing papaya production. When PRSV was detected in 1992 in Puna, the area produced 53 million pounds of fresh marketable papaya. PRSV-infected plants resulted in a steady decline in production such that Puna in 1998 produced only 26.7 million pounds of papaya. This is the year the seeds from transgenic papaya were released to growers. Production rebounded and in 2001, Puna produced 40 million pounds of fresh, marketable papaya (Table 1).

Table 1. Fresh papaya production[a] in state of Hawaii and in Puna district from 1992 to 2001.

Year	Total	**Puna** (x 1,000 lb)
(PRSV enters Puna) 1992	55,800	53,010
1993	58,200	55,290
1994	56,200	55,525
1995	41,900	39,215
1996	37,800	34,195
1997	35,700	27,810
(transgenic seeds released) 1998	35,600	26,750
1999	39,400	25,610
2000	50,250	33,950
2001	52,000	40,000

[a] Data were compiled from USDA Statistical Reports of Papaya grown in Hawaii (9).

Japanese market requires nontransgenic papaya. Japan has been a lucrative market for Hawaii's papaya for many years; in 1992 approximately 35% of Hawaii's papaya were exported to Japan. Unlike the USA mainland, transgenic papaya cannot be sold in Japan because efforts to deregulate the papaya in Japan are not yet completed. Thus, Hawaii needed to maintain its market in Japan and efforts to produce nontransgenic papaya have been ongoing since 1992. Soon after the discovery of PRSV in Puna in 1992, new plantations were started on different areas of Hawaii Island where PRSV had not been identified. Although these areas did not have the virus, Kapoho variety did not adapt well to these regions in that the fruit were generally smaller than those grown in Puna. The result was papaya production continued to drop and Hawaii began to lose market share in mainland USA and it became more difficult to maintain the shipment of quality nontransgenic papaya to Japan.

Transgenic papaya helps growers produce nontransgenic papaya in Puna. With the availability of the resistant Rainbow variety, and with the increased demand for nontransgenic papayas for the Japanese market, growers explored options for strategically deploying the newly deregulated transgenic Rainbow in an effort to assure economic yields of the nontransgenic Kapoho variety which were targeted for sale in Japan. This plan was developed by the HDOA, and was seen as an adjunct to their PRSV management program that called for surveying and roguing of infected trees monthly. In the HDOA plan, a 1,000-acre parcel of land in Kahuwai, which was isolated and predominantly upwind from the main planting areas in Puna, was targeted for nontransgenic fruit production destined for sale in Japan. About 600 acres were devoted to the production of nontransgenic Kapoho variety, and about 300 acres of the transgenic Rainbow was planted to create a buffer of resistant plants. The transgenic plants served to interrupt the movement of PRSV by viruliferous aphids into Kahuwai. The goal of this strategy was to reduce initial infection rates and secondary virus spread, thus slowing the PRSV epidemic in the Kahuwai management area. PRSV incidence in Kahuwai and in the remaining areas of Puna was monitored on a monthly basis by HDOA. HDOA surveyors marked infected trees, and growers were expected to rogue the trees within 5 to 7 days. If a grower failed to rogue trees, provisions in their leases called for possible termination of the lease.

Comparative PRSV disease progress data in 1999 for the Kahuwai management area and the remaining areas in Puna, and the 1992 PRSV data in Puna are presented in Fig. 1. While strict comparative experimental data were not available, the Kahuwai management area had very low PRSV incidence and represents the situation where a degree of isolation was possible, and roguing of infected plants was strictly followed. In the remaining areas of Puna, the disease incidence was much higher than Kahuwai. The likely reasons are that PRSV management was less intense and the more random planting of Rainbow was less effective in protecting nontransgenic plantings. HDOA, however, removed infected fields that had been abandoned by growers. This activity greatly reduced PRSV incidence and has served to keep the incidence of PRSV in nontransgenic papaya relatively low as compared with 1992-1998 when PRSV-resistant papaya were not available. The 1992 incidence data of PRSV in Puna represents the situation where roguing by growers was minimal since most growers did not believe that removing infected trees would be beneficial, and where transgenic papaya were not available.

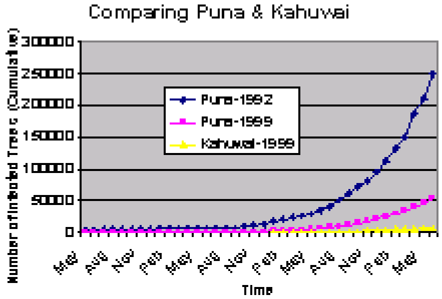

Fig. 1. Comparative PRSV disease progress curves for the Kahuwai PRSV management project area and the remaining areas of Puna from 1999. Superimposed is PRSV incidence data from the original 1992 PRSV outbreak in Puna. For the 1992 and 1999 Puna data, incidence are for approximately 2,200 acres and can be directly compared. The Kahuwai PRSV incidence data are for approximately 1,000 acres. This should be considered in making the relative comparisons.

These results (Fig. 1) suggest that this strategy of isolation and roguing successfully reduced the disease progress rate for PRSV in Kahuwai as compared to the remaining Puna region. They suggest that a strategy of planting an "island" of nontransgenic variety in an "ocean" of transgenic Rainbow might be useful for the economical production of the nontransgenic variety. Essential to the success of the Kahuwai project was the fact that a single landowner controlled the entire parcel and was willing to impose strict roguing of infected trees. As a result, the area was managed as if it were a single farm. In the remaining areas in Puna, the land was controlled by several different landowners who did not all agree to impose strict PRSV management guidelines within the terms of their leases to the growers. Consequently, it was not possible to impose the planting of strategic "islands" of the nontransgenic Kapoho variety within "oceans" of Rainbow. As a result, the PRSV epidemic progressed at a more rapid rate, approaching rates observed with the original outbreak of PRSV in Puna in 1992 (Fig. 1).

Concluding Remarks

The PRSV-resistant transgenic papaya has been commercially grown in Hawaii since 1998, and has played the most major role in saving the papaya industry from economical demise. The resistance has held up extremely well in Hawaii. The transgenic papaya case also illustrates the importance of developing control measures in advance of anticipated problems. Lastly, the case in Hawaii also shows that the transgenic papaya has helped growers to raise nontransgenic papaya in Puna by reducing the overall virus pressure in Puna and serving as buffer zones. In summary, the observations suggest that virus-resistant transgenic crops can directly control the virus and also serve as a tool to minimize infection to nontransgenic crops that are grown the area.

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